

high-fat diet (diet number D12451, containing 45% of calories as fat (Research Diets, New Brunswick, NJ)) for 6 weeks. After the 6 weeks, groups of mice received either vehicle (hydroxymethylcellulose), BI, BL, Wy14,643 or rosiglitazone by oral gavage at the indicated doses for an additional 4 weeks while continuing on the high-fat diet. Plasma chemistries (Anilytics, Inc., Gaithersburg, MD) were assayed after 2 weeks of drug treatments. Plasma serum insulin (Figure 1) and leptin (Figure 2) were measured by an electrochemiluminescent immunoassay (Origen Analyzer, Igen, Inc., Gaithersburg, MD) after 4 weeks of drug treatments.

- 10 BI and BL were effective at lowering serum triglycerides and free fatty acids as well as insulin and leptin serum levels. Serum values from mice from the same cohort ("lean controls") that were maintained on regular lab chow (Formulab Diet 5008, Quality Lab Products, Elkridge, MD) are shown for comparison.

Table 16:

	Triglycerides (mg/dL)	Free Fatty Acids (umol/L)
Vehicle	135 ± 40.1	1686 ± 359.3
BI (10 mg/kg)	68.8 ± 5.7	1227 ± 193.7
" (30 mg/kg)	66.5 ± 14.7	1292 ± 231.4
" (100 mg/kg)	37.4 ± 8.3	992.8 ± 172.1
BL (10 mg/kg)	80 ± 12.2	1571.8 ± 100.9
" (30 mg/kg)	66.4 ± 13.7	1413.2 ± 228.7
" (100 mg/kg)	41 ± 5.6	1133.5 ± 132.7
Rosiglitazone (1 mg/kg)	76.6 ± 16.5	1537 ± 256.3
" (3 mg/kg)	103.2 ± 10.8	1833.2 ± 169.8
" (10 mg/kg)	129.5 ± 48.7	1810.3 ± 595
" (100 mg/kg)	88 ± 7.2	1568.5 ± 197
Wy14643 (10 mg/kg)	70.6 ± 10.8	1512.2 ± 172.9
" (30 mg/kg)	88 ± 12.5	1676 ± 237
" (100 mg/kg)	88.4 ± 18.8	1839.8 ± 154.8
Rosi (3 mg/kg) + Wy14643 (100 mg/kg)	54.3 ± 10.5	1649.7 ± 260.5

EXAMPLE M: Oral BI lower circulating triglycerides, free fatty acids, insulin and leptin in high fat-fed Sprague Dawley rats

The high fat-fed rat is a model for insulin and leptin resistance. Sprague-Dawley rats have an intact leptin system and respond to a high fat diet with hyperinsulinemia due to a downregulation of the normal insulin response in peripheral tissues such as liver, adipose tissue and muscle

Male Sprague-Dawley rats, approximately 17 weeks of age, were obtained from Jackson Labs (Bar Harbor, ME) and randomly assigned into groups of 5 – 7 animals; the body weights were similar between groups. All animals were maintained in a temperature-controlled (25°C) facility with a strict 12 h light/dark cycle and were given free access to water and food. Rats were fed a high-fat diet (diet number D12451 (containing 45 % of calories as fat), Research Diets, New Brunswick, NJ) for one month prior to drug treatment.

Groups of 6 Sprague-Dawley rats were treated with a single daily dose of vehicle (hydroxymethylcellulose), BI (10, 30 and 100 mg/kg), or rosiglitazone (3 mg/kg) for 6 weeks while maintaining the high-fat diet. At the indicated time points, blood samples (~100 µl) were obtained via the tail vein for serum chemistry analysis.

BI (30 mg/kg) reduced serum insulin, triglycerides; BI at all doses reduced free fatty acids.

Table 17: Effect of BI and rosiglitazone on serum glucose, insulin, triglycerides and free fatty acids in high-fat fed Sprague-Dawley rats

Group	Glucose (mg/dL)	Insulin (ng/ml)	Triglycerides (mg/dL)	Free Fatty Acids (µMol/L)
Lean	123.8 ± 7.0	0.72 ± 0.1	179.0 ± 72.3	743.5 ± 57.4
Vehicle	122.3 ± 5.9	1.78 ± 0.3	200.7 ± 39.2	942.5 ± 181.0
BI-10	117.3 ± 8.8	2.18 ± 0.9	183.7 ± 58.4	923.7 ± 161.3
BI-30	127.3 ± 22.2	1.46 ± 0.2	129.3 ± 20.0	738.7 ± 50.0
BI-100	19.3 ± 3.5	1.79 ± 0.2	171.7 ± 33.1	725.7 ± 87.5
RG-3	119.8 ± 5.4	1.57 ± 0.2	134.2 ± 15.2	758.8 ± 61.0